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Liquid Chromatography – Mass Spectrometry (LC-MS) Analysis and Antifungal Potential of *Cyperus iria, Fimbristylis miliacea,* and *Fimbristylis globulosa*

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The plants from the Cyperaceae family have been long used for natural remedies including biopesticides to treat plant pathogens. The species *Cyperus iria*, *Fimbristylis miliacea*, and *Fimbristylis globulosa* contain bioactive compounds such as flavonoids and terpenoids that can be used to treat plant disease including fungal infection on plants. However, there is no reported data on the antifungal potential of the plants on *Colletotrichum* and *Fusarium* species, nor phytochemical studies conducted on the species. This study investigates the phytochemical contents of *C. iria, F. miliacea,* and *F. globulosa* methanolic extracts using liquid chromatography-mass spectrometry (LC-MS) technique, and their antifungal activity using agar well diffusion method and Minimum Inhibitory and Fungicidal Concentration (MIC & MFC). A total of 131, 60 and 95 compounds mainly from flavonoids, terpenes and fatty acids classes have been detected in the *C. iria, F. miliacea*, and *F. globulosa* extracts, respectively. The antifungal activity of the extracts against *Colletotrichum* spp. showed moderate to good activity with MIC and MFC values of 125 ug/mL and 250 ug/mL, respectively. Their antifungal activity against *Fusarium* spp. also showed moderate to good activity with MIC and MFC value of 250 ug/mL for *C. iria* and *F. miliacea*, and moderate activity for *F. globulosa* with MIC value of 250 ug/mL and MFC value of 500 ug/mL. These findings were supported by previous studies that Cyperaceae contain secondary metabolites which showed potential antifungal activity of Cyperaceae.

Keywords: Antifungal; Cyperaceae; LC-MS; phytochemical; plant extracts

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The Cyperaceae is a family of graminoid (grass-like), monocotyledonous flowering plants known as sedges, which superficially resemble the closely related rushes and the more distantly related grasses. The family is large, with some 5,500 known species described in about 90 genera [1-3]. They can be found in Tropical Asia and Tropical South America [4]. While sedges may be found growing in almost all environments, many are associated with wetlands, or with poor soils. Many sedges are used as foods, food additives, drinks, fibers, animal poisons, and in the manufacturing of items including paper, perfumes, medicines, mats, boats, clothing, shoes, ropes, and roofing.

The species from this family have been proven to exert various biological activities such as antioxidant, antimicrobial, anti-inflammatory, anticancer and many more [5, 6]. This is due to the presence of biological active classes of compounds including flavonoids, terpenoids, phenolics, essential oils, alkaloids, tannins, and saponins [7]. Among the classes of bioactive compounds reported from the Cyperaceae species, flavonoids and terpenoids were proven to be associated with antifungal activity [7]. In this study, we have selected two genera from the Cyperaceae family which are *Cyperus* and *Fimbristylis*. The previous study on the genus *Cyperus* reported the existence of stilbenoids, flavonoids, triterpenes, and other compounds. However, there is limited study on phytochemical compounds on the genus of *Cyperus* and no work has been done on *Fimbristylis.* In Malaysia, Cyperaceae can be found in wetland areas and paddy fields [4]. The presence of this weed has caused a nuisance to the farmers as it requires chemical herbicide to eradicate the weed. This however may raise safety concerns among the consumer as we all know that rice is a staple food in Malaysia. Thus, the presence of plant metabolites in this species makes it possible to be applied as biopesticides.

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Colletotrichum spp. and *Fusarium* spp. are two most important plant pathogens which can infect a variety of hosts such as grains, legumes, cereals, vegetables, and fruits [8, 9]. *Colletotrichum* spp. is the pathogen which can cause anthracnose disease [10]. It is the causing agents of anthracnose which are able to cause irregular circular spots on leaves, invade the shortened crown and expand into an annular ring, causing the host to be unable to absorb water and nutrients and eventually killing the host [10, 11]. While *Fusarium* spp. is able to cause bakanae disease in rice [8]. It can be mainly found in soil. Mycotoxins were produced by some of the species in this genus which may affect cereal crops and animal health if they enter the food chain [8]. This present study has been undertaken to determine the chemical constituents of the methanolic extract from *C. iria*, *F. miliacea*, and *F. globulosa* using LC-MS and correlate them to their in vitro antifungal activities against *Colletotrichum* spp. and *Fusarium* spp.

EXPERIMENTAL

Sample Extraction

Three plant samples; *C. iria*, *F. miliacea*, and *F. globulosa* were collected from Merlimau, Melaka, Malaysia (2. 1812° N, 102.4266° E) from September 2022 until December 2022. The sample was identified by a botanist Dr Shamsul Khamis from UKM. The voucher numbers for the plant are ID 073/2023 (*C. iria*), ID 072/2023 (*F. miliacea*), and ID 071/2023 (*F. globulosa*). The samples were cleaned and oven dried in a weed laboratory in UiTM Melaka, Campus Jasin for 2-3 days and whole plant parts were grinded into fine powder using a heavy-duty grinder (Retsch GmbH, Germany). The samples were extracted using a maceration method described by Rukayadi et al. (2008), with slight modification. 100 g of powdered samples were soaked in 500 ml of methanol (HmbG Chemicals) and left for three days at room temperature. The mixtures were filtered using Whatman filter paper No. 1 (Whatman International Ltd.) and further concentrated using a rotary evaporator at 60°C.

Liquid Chromatography- Mass Spectrometry (LC-MS) Analysis

Instrument

The LC/MS-QTOF system used in this study comprised of an Agilent 1200 liquid chromatography system, equipped with a binary pump, a vacuum degasser unit, an auto sampler and 6520 quadrupole time of flight mass spectrometer with an electrospray ionization (ESI) source in positive mode with optimum gas temperature at 325°C, gas flow at 11 L/min and nebulizer at 35 psi.

Chromatography and Mass Spectrometry Method

Chromatographic separation was performed at 40°C using Agilent ZORBAX Eclipse Plus C18 Rapid Resolution HT (2.1 x 100 mm) 1.8 µm with (A) 0.1% formic acid in ultra-pure H_2O and (B) 0.1% formic acid in acetonitrile. The gradient elution program was 0.00 – 18.00 min, 5 -95%(B); 18 to 23 minutes; 95% (B). 23.01 minutes; 5% (B). The total run time was 30 minutes. The LC condition was re-equilibrated for 2 minutes before starting the new injection. The sample injection volume was set at 2 µl and the flow rate of the mobile phase was set at 0.25 mL/min.

Sample Preparation

The 1 mg/mL sample was prepared and vortexed for 60 seconds. The sample was then centrifuged at 10,000 RPM for 10 minutes, and the supernatant was filtered using a 0.22 µm pore size syringe filter before analysis.

Data Analysis

Agilent MassHunter Qualitative Analysis B.05.00 software (Agilent Technologies, Santa Clara, CA, USA) was used for the data analysis (MS data (.d)). The chromatographic profiles were analysed based on the accurate mass data identified and the predicted compounds were annotated using Europian Bio-informatics Institute, Human Metabolome Database, National Center for Biotechnology Information, National Library of Medicine, FooDB, and METLIN database.

Antifungal Activity

Colletotrichum spp. and *Fusarium* spp., isolated from the plant pathogen were obtained from Pathology Lab in Faculty Plantation and Agrotechnology, UiTM Melaka, Campus Jasin. The fungal were maintained on potato dextrose agar (PDA) in the incubator for seven days. This procedure was done using the method from Aneja and Joshi (2009) and Zain, et al (2019). Ten mL of the potato dextrose agar (PDA) were poured into petri dishes and were allowed to solidify. The wells were prepared in the plates using cork borers with the diameter of 5 mm. Then, 20 µL of microorganism and compounds were pipetted into the well directly. This step was repeated for both extracts and control. Control used was Kencozeb M45 and it was prepared by dissolving 0.025 g of Kencozeb M45 powder in 10 mL distilled water. The plates were incubated at 37 °C. All tests were made in three replications. The zones of inhibition (ZoI) around the discs will be measured after 24/48/72 hours of incubation and the results were analysed statistically.

Table 1. Compounds identified using LC-MS analysis from *C. iria*, *F. miliacea*, and *F. globulosa*.

Isolated compounds	Plant sample			Molecular formula	*(m/z)*	Class of compound	Reference
	C. iria	*F. miliacea*	*F. globulosa*	$C_7H_4Cl_2O_3$	223.9885	Carboxylic Acid	National Center for Biotechnology Information, 2005
3,5-Dichlorosalicylic acid	√			$C_{12}H_{23}NO_{10}$	342.1389	Monosaccharides	METLIN Database
6-(Alpha-D-Glucosaminyl)-1D-myo-inositol	√			$C_{19}H_{18}O_6$	381.0723	Organic Cyclic	National Center for Biotechnology Information, 2009
5,7,2',5'-Tetramethoxyflavone	√			$C_{20}H_{16}O_4S$	295.1099	Benzenoids	National Center for Biotechnology Information, 2005
7-Hydroxymethyl-12-methylbenz[a]anthracene	√			$C_{26}H_{28}O_7$	475.1738	Pyranoxanthones	National Center for Biotechnology Information, 2009
Artonin S	√			$C_{17}H_{30}O_{13}$	460.2009	Oligosaccharides	FooDB, 2010
Beta-D-xylopyranosyl-(1-4)-alpha-L-rhamnopyranosyl-(1-2)-D-fucose	√			$C_{11}H_{21}NO_7$	280.1382	Organonitrogen	National Center for Biotechnology Information, 2017
Mycosporine	√			$C_{11}H_{19}NO_6$	262.1274	Amino acid	METLIN Database
N-hydroxy-L-phenylalanine	√		√	$C_9H_{11}NO_3$	182.0804	Organonitrogen	National Center for Biotechnology Information, 2007
Betaine	√	√		$C_5H_{12}NO_2$	118.0859	Organonitrogen	National Center for Biotechnology Information, 2004
Vidarabine	√			$C_{10}H_{13}N_5O_4$	268.1034	Organic Heterocyclic	National Center for Biotechnology Information, 2005
Triethyl citrate	√			$C_{12}H_{20}O_7$	294.1544	Organochaclogen	National Center for Biotechnology Information, 2005
L-Leucine	√			$C_6H_{13}NO_2$	132.1013	Amino acid	National Center for Biotechnology Information, 2004
N-(1-Deoxy-1-fructosyl)valine	√	√	√	$C_{11}H_{21}NO_7$	280.1386	Organonitrogen	National Center for Biotechnology Information, 2017
m-Coumaric acid	√			$C_9H_8O_3$	182.0809	Organic Acid	National Center for Biotechnology Information, 2005
1,1'-(Tetrahydro-6a-hydroxy-2,3a,5-trimethylfuro[2,3-d]-1,3-dioxole-2,5-diyl)bis-ethanone	√			$C_{12}H_{18}O_6$	276.1441	Flavonoids	National Center for Biotechnology Information, 2005
N-(1-Deoxy-1-fructosyl)isoleucine	√			$C_{12}H_{23}NO_7$	294.1543	Amino acid	National Library of Medicine, 2017
N,N-Diethylglycine	√	√	√	$C_6H_{13}NO_2$	132.1016	Amino acid	National Library of Medicine, 2005
N-(1-Deoxy-1-fructosyl)phenylalanine	√		√	$C_{15}H_{21}NO_7$	328.1386	Amino acid	National Library of Medicine, 2015

Tranylcypromine glucuronide	√			$C_{15}H_{19}NO_6$	310.1283	Monoamine	METLIN Database
3-Isochromanone	√			$C_9H_8O_2$	166.0856	Benzopyrans	METLIN Database
3-Amino-2-naphthoic acid	√		√	$C_{11}H_9NO_2$	205.0975	Carboxylic acid	National Library of Medicine, 2005
1,4,5,8-Tetraaminoanthraquinone	√			$C_{14}H_{12}N_4O_2$	291.0859	Anthraquinones	National Library of Medicine, 2005
Gambiriin B3	√			$C_{30}H_{26}O_{11}$	563.1543	Flavonoids	National Library of Medicine, 2010
Cyanidin 3-(2"-glucuronosylglucoside)	√			$C_{27}H_{29}O_{17}$	625.1402	Flavonoids	METLIN Database
Delhpinidin 3-neohesperidoside	√			$C_{27}H_{31}O_{16}$	611.1612	Flavonoids	National Center for Biotechnology Information, 2005
6-Hydroxycyanidin 3-glucoside	√			$C_{21}H_{21}O_{12}$	465.1031	Flavonoids	METLIN Database
Leucodelphinidin 3-[galactosyl-(1-4)-glucoside]	√			$C_{27}H_{34}O_{18}$	669.1621	Flavonoid o-glycosides	(FooDB, 2010)
Cearoin	√			$C_{14}H_{12}O_4$	245.0806	Ketones	National Library of Medicine, 2005
4-(2-Hydroxypropoxy)-3,5-dimethyl-phenol	√	√	√	$C_{11}H_{16}O_3$	197.1165	Flavonoids	METLIN Database
Ishwarol	√			$C_{15}H_{24}O$	221.1893	Sesquiterpene	National Library of Medicine, 2009
Patchoula-2,4-diene	√			$C_{15}H_{22}$	203.1788	Sesquiterpene	METLIN Database
2,3,4,5,2',3',4',6'-Octamethoxychalcone	√			$C_{23}H_{28}O_9$	487.1362	Flavonoids	METLIN Database
Aurantinidin	√			$C_{15}H_{11}O_6$	287.0548	Benzopyran	National Library of Medicine, 2005
Petunidin	√			$C_{16}H_{13}O_7$	317.0671	Anthocyanin	National Library of Medicine, 2005
Seiricardine A	√			$C_{15}H_{26}O_2$	239.2000	Sesquiterpenes	National Library of Medicine, 2005
1,2,4,5-Tetramethylbenzene	√			$C_{10}H_{14}$	135.1166	Benzene	National Library of Medicine, 2005
Lyngbic acid	√			$C_{15}H_{28}O_3$	279.1936	Phenolic acid	National Library of Medicine, 2006
Malvidin	√	√	√	$C_{17}H_{15}O_7$	331.0811	Anthocyanin	National Library of Medicine, 2005
Viscutin 1	√		√	$C_{27}H_{26}O_{11}$	527.1562	Organooxygen	National Library of Medicine, 2009
2",6"-Di-O-acetylononin	√	√	√	$C_{26}H_{26}O_{11}$	515.1541	Isoflavonoids	METLIN Database
11*Z*-Tridecenyl acetate	√			$C_{15}H_{28}O_2$	241.2161	Monoterpenoids	METLIN Database
6-Desmethoxy hormothamnione triacetate	√			$C_{27}H_{26}O_{11}$	527.1547	Chalcone	METLIN Database
Corynebactin	√			$C_{39}H_{42}N_6O_{18}$	900.2907	Amino acid	National Library of Medicine, 2005
Isophylloflavanine	√		√	$C_{35}H_{32}O_{13}$	661.1932	Isoflavonoids	METLIN Database

Patchoula-2,4-diene	√			$C_{15}H_{22}$	203.1785	Monoterpenoids	METLIN Database
Phellatin	√	√		$C_{26}H_{30}O_{12}$	557.1635	Tannins	National Library of Medicine, 2009
10-Tridecynoic acid	√	√		$C_{13}H_{22}O_2$	228.1950	Fatty Acid	National Library of Medicine, 2006
2,6-Nonadien-1-ol	√			$C_9H_{16}O$	158.1540	Fatty Alcohol	National Library of Medicine, 2005
C17-Sphinganine	√	√		$C_{17}H_{37}NO_2$	288.2893	Organic Amino	National Library of Medicine, 2014
4-Vinylcyclohexene	√			C_8H_{12}	109.1010	Hydrocarbons	National Library of Medicine, 2005
3(4-5)-Abeo-4,11:4,12-diepoxy-3-eudesmanol	√			$C_{15}H_{24}O_3$	253.1795	Organic Heterocyclic	National Library of Medicine, 2014
(*R*)-Camphor	√			$C_{10}H_{16}O$	153.1270	Monoterpene	METLIN Database
4,8,12,16-Hexadecatetraenoic acid	√			$C_{16}H_{24}O_2$	249.1847	Fatty Acid	National Library of Medicine, 2006
Juvenile hormone III	√			$C_{16}H_{26}O_3$	267.1951	Sesquiterpenes	National Library of Medicine, 2005
(+)-Mayurone	√			$C_{14}H_{22}O$	207.1739	Organooxygen	National Library of Medicine, 2007
cis-Caryophyllene	√			$C14H_{22}$	191.1789	Sesquiterpenoids	METLIN Database
4,8-dimethylnonanoyl carnitine	√			$C_{18}H_{36}NO_4$	330.2642	Fatty Acid	National Library of Medicine, 2011
Curcumenol	√		√	$C_{15}H_{22}O_2$	235.1686	Sesquiterpenoid	METLIN Database
Furodysinin	√			$C_{15}H_{20}O$	217.1584	Terpenoid	National Library of Medicine, 2005
(10*S*)-Juvenile hormone III diol	√			$C_{16}H_{28}O_4$	307.1871	Sesquiterpenoid	National Library of Medicine, 2008
4,5-Di-O-methyl-8-prenylafzelechin-4beta-ol	√		√	$C_{22}H_{26}O_6$	387.1802	Flavonoids	National Library of Medicine, 2009
Ishwarol	√			$C_{15}H_{24}O$	221.1889	Fatty Acid	National Library of Medicine, 2005
Plakortic acid	√			$C_{17}H_{30}O_4$	321.2032	Organic Heterocyclic	National Library of Medicine, 2006
Phytosphingosine	√	√		$C_{18}H_{39}NO_3$	318.3004	Organic Amino	National Library of Medicine, 2005
6-Oxabicyclo[3.1.0]hexane-2-undecanoic acid methyl ester	√			$C_{17}H_{30}O_3$	283.2268	Fatty Acid	National Library of Medicine, 2005
13-methyl-octadecanoic acid	√	√	√	$C_{19}H_{38}O_2$	316.3216	Fatty Acid	National Library of Medicine, 2006
Seiricardine A	√			$C_{15}H_{26}O_2$	261.1815	Sesquiterpenoids	National Library of Medicine, 2005
Cetrimide	√			$C_{17}H_{38}N$	256.2987	Amine	National Library of Medicine, 2005
(2*S*)-2-hydroxyphytanic acid	√			$C_{20}H_{40}O_3$	346.3311	Prenolic Lipid	National Library of Medicine, 2004
Kakuol	√			$C_{10}H_{10}O_4$	195.0650	Ketones	National Library of Medicine, 2005

4,8-Diacetyl-T2-tetrol	√			$C_{19}H_{26}O_8$	405.1534	Sesquiterpenes	FooDB, 2010
1-Linoleoyl-2-(1-enyl-stearoyl)-sn-glycero-3-phosphocholine	√			$C_{26}H_{51}NO_7P$	520.3405	Fatty Acid	METLIN Database
Furodysinin	√			$C_{15}H_{20}O$	217.1582	Terpenoids	National Library of Medicine, 2005
3α-Hydroxy-4,4-bisnor-8,11,13-podocarpatriene	√			$C_{15}H_{20}O$	217.1588	Diterpenoids	METLIN Database
Plakortic acid	√			$C_{17}H_{30}O_4$	321.2041	Organic Heterocyclic	National Library of Medicine, 2006
10,11-Epoxy-3,7,11-trimethyl-2*E*,6*E*-tridecadienoic acid	√			$C_{16}H_{26}O_3$	267.1959	Monoterpenoid	National Library of Medicine, 2005
8*E*-Tetradecenyl acetate	√			$C_{16}H_{30}O_2$	277.2145	Fatty Acid	National Library of Medicine, 2005
1-Monopalmitin	√			$C_{19}H_{38}O_4$	353.2663	Fatty Acid	METLIN Database
4,8,12,16-Hexadecatetraenoic acid	√			$C_{16}H_{24}O_2$	249.1841	Fatty Acid	National Library of Medicine, 2006
Juvenile hormone III	√			$C_{16}H_{26}O_3$	267.1954	Sesquiterpenes	National Library of Medicine, 2005
3,5,6,7,8,4'-Hexamethoxyflavone	√			$C_{21}H_{22}O_8$	403.1375	Flavonoids	METLIN Database
Malyngamide H	√		√	$C_{26}H_{41}NO_4$	454.2934	Monoterpenoid	National Library of Medicine, 2006
1-Tetradecyl-2-acetyl-sn-glycero-3-phosphocholine	√			$C_{24}H_{51}NO_7P$	496.3383	Fatty Acid	National Library of Medicine, 2006
9*Z*,12*Z*,15*E*-Octadecatrienoic acid	√	√	√	$C_{18}H_{30}O_2$	279.2314	Fatty Acid	National Library of Medicine, 2005
Avocadynofuran	√			$C_{17}H_{26}O$	285.1614	Organic Heterocyclic	National Library of Medicine, 2006
α-Hydroxy farnesyl phosphonic acid	√			$C_{15}H_{27}O_4P$	325.1546	Sesquiterpenoids	METLIN Database
1-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-1,6-heptadien-3-one	√			$C_{16}H_{24}O$	271.1457	Sesquiterpenoids	FooDB, 2010
Polysorbate 60	√	√		$C_{22}H_{42}O_8$	452.3233	Fatty Acid Esters	FooDB, 2010
10,11-Epoxy-3,7,11-trimethyl-2E,6E-tridecadienoic acid	√			$C_{16}H_{26}O_3$	267.1959	Monoterpenoid	National Library of Medicine, 2005
(S)-Beta-himachalene	√			$C_{15}H_{24}$	205.1954	Sesquiterpene	National Library of Medicine, 2008
Trachelogenin	√			$C_{21}H_{24}O_7$	389.1602	Phenylpropanoids	National Library of Medicine, 2005
Minosaminomycin	√		√	$C_{25}H_{46}N_8O_{10}$	619.3405	Carbohydrates	National Library of Medicine, 2005
Cyperolone	√			$C_{15}H_{24}O_2$	237.1840	Sesquiterpenes	National Library of Medicine, 2006
Santene	√			C_9H_{14}	123.1170	Terpenoids	National Library of Medicine, 2005

Emmotin A	√	√	√	$C_{16}H_{22}O_4$	279.1600	Sesquiterpenes	National Library of Medicine, 2009
1-Monoacylgylceride	√			$C_{21}H_{36}O_4$	353.2673	Glycerides	Europian Bioinformatics Institute, 2020
2,3-Dihydroxycyclopentaneundecanoic acid	√	√	√	$C_{16}H_{30}O_4$	287.2228	Fatty Acid	(National Center for Biotechnology Information, 2023)
1-(O-alpha-D-glucopyranosyl)-27-keto-(1,3R,29R)-triacontanetriol	√			$C_{36}H_{70}O_9$	685.4676	Fatty Acids	METLIN Database
3-Methyl-5-pentyl-2-furanundecanoic acid	√			$C_{21}H_{36}O_3$	337.2723	Fatty Acid	(National Center for Biotechnology Information, 2023)
5-(1-Hydroxypropan-2-yl)isolongifol-4-ene	√	√	√	$C_{18}H_{30}O$	263.2368	Sesquiterpenoid	(National Center for Biotechnology Information, 2023)
Palmitic amide	√	√	√	$C_{16}H_{33}NO$	256.2625	Fatty Acid	(National Center for Biotechnology Information, 2023)
Dodemorph	√	√	√	$C_{18}H_{35}NO$	282.2793	Organic Amino	(National Center for Biotechnology Information, 2023)
1-Monopalmitin	√	√	√	$C_{19}H_{38}O_4$	331.2828	Fatty Acid	METLIN Database
1,2-di-(9*Z*,12Z-octadecadienoyl)-sn-glycero-3-phosphoethanolamine	√			$C_{44}H_{84}NO_8P$	808.5838	Fatty Acid	(National Center for Biotechnology Information, 2023)
Astrophylline	√	√		$C_{19}H_{26}N_2O$	337.167	Lignan	(National Center for Biotechnology Information, 2023)
Heneicosanedioic acid	√		√	$C_{21}H_{40}O_4$	357.2992	Fatty Acid	(National Center for Biotechnology Information, 2006)
Dihydrotachysterol	√	√		$C_{28}H_{46}O$	421.3457	Triterpenoids	METLIN Database
2,4,12-Octadecatrienoic acid isobutylamide	√			$C_{22}H_{39}NO$	334.3103	Fatty Acid	(National Center for Biotechnology Information, 2009)
Squamocin L	√			$C_{37}H_{66}O_6$	629.4763	Organooxygen	(National Center for Biotechnology Information, 2005)
Pyrimidifen	√		√	$C_{20}H_{28}ClN_3O_2$	395.2209	Organic Amino	(National Center for Biotechnology Information, 2006)
N-stearoyl valine	√	√	√	$C_{23}H_{45}NO_3$	384.3456	Fatty Acid	(National Center for Biotechnology Information, 2007)
Stearamide	√		√	$C_{18}H_{37}NO$	284.2949	Fatty Acid	(National Center for Biotechnology Information, 2005)
Schleicherastatin 3	√			$C_{29}H_{50}O_3$	469.366	Steroids	(Human Metabolome Database, 2012)
Cholesteryl acetate	√			$C_{29}H_{48}O_2$	429.3733	Steroids	(National Center for Biotechnology Information, 2005)
1-Tetradecanoyl-2-(7Z,10Z,13Z,16Z,19Z-docosapentaenoyl)-sn-glycerol	√			$C_{35}H_{66}O_5$	589.4818	Fatty Acid	(National Center for Biotechnology Information, 2011)
1-(9Z-Tetradecenoyl)-2-(7Z,10Z,13Z,16Z,19Z-docosapentaenoyl)-*sn*-	√			$C_{39}H_{64}O_5$	613.4835	Fatty Acid	(National Center for Biotechnology Information, 2012)

glycerol							
10-Oxo-nonadecanoic acid	√		√	$C_{19}H_{36}O_3$	313.2732	Fatty Acid	(National Center for Biotechnology Information, 2006)
Glycerol 2-(9*Z*,12*Z*-octadecadienoate) 1-hexadecanoate 3-O-[alpha-D-galactopyranosyl-(1-6)-beta-D-galactopyranoside]	√			$C_{49}H_{88}O_{15}$	934.6449	Fatty Acid	(FooDB, 2010)
10,11-Epoxy-3,7,11-trimethyl-2*E*,6*E*-tridecadienoic acid	√			$C_{16}H_{26}O_3$	555.3667	Monoterpenoid	(National Library of Medicine, 2005)
Ganoderiol I	√			$C_{31}H_{50}O_5$	525.3547	Triterpenoid	(National Center for Biotechnology Information, 2007)
Euphornin	√			$C_{33}H_{44}O_9$	607.2888	Terpenoids	(National Center for Biotechnology Information, 2007)
4-Keto-γ-carotene/ Keto-γ-carotene	√		√	$C_{40}H_{54}O$	551.4249	Terpenoids	(National Center for Biotechnology Information, 2007)
1-Myristoyl-2-meadoyl-sn-glycerol	√			$C_{37}H_{66}O_5$	613.4809	Fatty Acid	(National Center for Biotechnology Information, 2011)
1,2-Di-(9Z,12Z,15Z-octadecatrienoyl)-3-(Galactosyl-alpha-1-6-Galactosyl-beta-1)-glycerol	√	√	√	$C_{51}H_{84}O_{15}$	954.6114	Organooxygen	(National Center for Biotechnology Information, 2011)
6*Z*,9*Z*-Eicosadien-11-ol	√		√	$C_{20}H_{38}O$	312.3255	Fatty Acid	METLIN Database
6-Deoxodolichosterone	√			$C_{28}H_{48}O_4$	471.3457	Terpenoids	(National Center for Biotechnology Information, 2007)
3α,12α-Dihydroxy-5β-chol-8(14)-en-24-oic Acid	√	√	√	$C_{24}H_{38}O_4$	391.2840	Fatty Acid	(National Center for Biotechnology Information, 2006)
Myricetin 3-O-(4"-O-acetyl-2"-O-galloyl)-alpha-L-rhamnopyranoside	√			$C_{30}H_{26}O_{17}$	676.1520	Flavonoids	METLIN Database
N-Cyclohexanecarbonylpentadecylamine	√	√	√	$C_{22}H_{43}NO$	338.3416	Fatty acid	National Center for Biotechnology Information, 2011
35-Aminobacteriohopane-31,32,33,34-tetrol	√			$C_{35}H_{63}NO_4$	562.4817	Terpenes	National Center for Biotechnology Information, 2015
1,2-Dichloropropane		√		$C_3H_6Cl_2$	130.018	Organochalcogen	National Center for Biotechnology Information, 2005
3-Methoxy-4,5-methylenedioxybenzoic acid		√		$C_9H_8O_5$	219.0270	Phenolic	Human Metabolome Database, 2012
Nigerose (Sakebiose)		√	√	$C_{12}H_{22}O_{11}$	381.0794	Organooxygen	National Center for Biotechnology Information, 2005
L-Galactose		√	√	$C_6H_{12}O_6$	203.0526	Organooxygen	National Center for Biotechnology Information, 2004
D-(+)-Cellobiose		√		$C_{12}H_{22}O_{11}$	365.1055	Organooxygen	National Center for Biotechnology Information, 2005

D-Proline		√		$C_5H_9NO_2$	116.0704	Organic Amino	National Center for Biotechnology Information, 2004
3-Hydroxy-3-methyl-glutaric acid		√	√	$C_6H_{10}O_5$	180.0856	Organic Hydroxy	National Center for Biotechnology Information, 2004
Arginyl-Proline		√	√	$C_{11}H_{21}N_5O_3$	294.1538	Organic Amino	National Center for Biotechnology Information, 2005
Quinacetol		√		$C_{11}H_9NO_2$	205.0970	Alkaloids	METLIN Database
Cyanidin 5-O-glucoside		√	√	$C_{21}H_{21}O_{11}$	449.1075	Flavonoids	National Center for Biotechnology Information, 2007
Malvidin 3-rutinoside		√	√	$C_{29}H_{35}O_{16}$	639.1938	Anthocyanidin	National Center for Biotechnology Information, 2009
Oenin		√	√	$C_{23}H_{25}O_{12}$	493.1347	Flavonoids	National Center for Biotechnology Information, 2005
Diospyrin		√		$C_{21}H_{24}O_{13}$	485.1272	Organic Hydroxy	National Center for Biotechnology Information, 2005
9,10-Epoxyoctadecatrienoic acid		√		$C_{18}H_{28}O_3$	293.2105	Fatty Acid	National Center for Biotechnology Information, 2007
Malvidin 3- (6"-p-coumarylglucoside) -5-(2'"-acetylxyloside)		√		$C_{39}H_{41}O_{19}$	813.2202	Flavonoids	METLIN Database
6-Desmethoxy hormothamnione diacetate		√		$C_{25}H_{24}O_{10}$	485.1439	Chalcone	METLIN Database
5*Z*,8*Z*,11*Z*,14*Z*-Octadecatetraenoic acid		√	√	$C_{18}H_{28}O_2$	277.2157	Fatty Acid	National Center for Biotechnology Information, 2006
N,N,N-trimethyl-sphingosine		√	√	$C_{21}H_{44}N\ O_2$	293.2117	Fatty Acid	National Center for Biotechnology Information, 2007
13-Beta-D-Glucosyloxydocosanoate		√		$C_{28}H_{54}O_8$	342.3374	Fatty Acid	National Center for Biotechnology Information, 2006
2-monorglyceride		√		$C_{21}H_{36}O_4$	353.2680	Organochalcogen	Europian Bioinformatics Institute, 2022
Pipericine		√		$C_{22}H_{41}NO$	336.3260	Fatty Acid	National Center for Biotechnology Information, 2006
4*Z*-Decenyl acetate		√		$C_{12}H_{22}O_2$	199.1684	Fatty Acid	National Center for Biotechnology Information, 2005
10*E*,12*E*,14*E*-Hexadecatrienyl acetate		√		$C_{18}H_{30}O_2$	279.2306	Fatty Acid	National Center for Biotechnology Information, 2007
1-Linoleoyl Glycerol		√		$C_{21}H_{38}O_4$	355.2830	Fatty Acid	METLIN Database
Kanokoside D		√		$C_{27}H_{44}O_{16}$	625.2692	Terpenoid	National Center for Biotechnology Information, 2010
6*E*,9*E*-Octadecadienoic acid		√	√	$C_{18}H_{32}O_2$	281.2471	Lipid (Fatty Acid)	Europian Bioinformatics Institute, 2023
Dihydrostreptomycin 6-phosphate		√		$C_{28}H_{46}O$	702.2117	Organic Hydroxy	National Center for Biotechnology Information, 2004

15Z-Docosenoic acid		√	√	$C_{22}H_{42}O_2$	339.3266	Fatty Acid	National Center for Biotechnology Information, 2006
Prostaglandin f1alpha		√		$C_{20}H_{36}O_5$	395.2183	Fatty Acid	National Center for Biotechnology Information, 2005
Stearamide		√		$C_{18}H_{37}NO$	284.2947	Fatty Acid	National Center for Biotechnology Information, 2005
1-Monoacylglyceride		√		$C_{27}H_{52}O_4$	441.3934	Fatty Acid	Human Metabolome Database, 2009
Lansioside C		√	√	$C_{35}H_{56}O_7$	589.4095	Organochalcogen	National Center for Biotechnology Information, 2014
Docosanedioic acid		√	√	$C_{22}H_{42}O_4$	371.3152	Fatty Acid	National Center for Biotechnology Information, 2005
Tetracosanedioic acid		√		$C_{24}H_{46}O_4$	416.3732	Fatty Acid	National Center for Biotechnology Information, 2005
Glycyl-glycine			√	$C_4H_8N_2O_3$	133.0605	Organic Amino	National Center for Biotechnology Information, 2004
3-Acetamidopropanal			√	$C_5H_9NO_2$	116.0703	Oganonitrogen	National Center for Biotechnology Information, 2004
Cortisol 21-sulfate			√	$C_{21}H_{30}O_8S$	460.1989	Fatty Acid	National Center for Biotechnology Information, 2004
Isoamyl nitrite			√	$C_5H_{11}NO_2$	118.0864	Nitrites	National Center for Biotechnology Information, 2005
Pyroglutamic acid			√	$C_5H_7NO_3$	130.0503	Organic Amino	National Center for Biotechnology Information, 2004
Miserotoxin			√	$C_9H_{17}NO_8$	268.1028	Organochalcogen	National Center for Biotechnology Information, 2005
Gambiriin B2			√	$C_{30}H_{26}O_{11}$	563.1567	Organic Hydroxy	National Center for Biotechnology Information, 2007
Peonidin 3-galactoside			√	$C_{22}H_{23}O_{11}$	463.1238	Anthocyanidin	METLIN Database
Guibourtinidol-4alpha-ol			√	$C_{15}H_{14}O_5$	275.0912	-	METLIN Database
Guibourtinidol-(4alpha-6)-catechin			√	$C_{30}H_{26}O_{10}$	547.1605	Phenols (Tannin)	National Center for Biotechnology Information, 2005
Rhamnetin 3-rhamnosyl-(1-3)(4'''-acetylrhamnosyl)(1-6)-galactoside			√	$C_{36}H_{44}O_{21}$	835.2267	Flavonoids	METLIN Database
Acacetin 7-O-[6"-O-glucosyl-2"-O-(3'''-acetylrhamnosyl)glucoside			√	$C_{36}H_{44}O_{20}$	819.2292	Flavonoids	METLIN Database
Ryanodine			√	$C_{25}H_{35}NO_9$	494.2375	Alkaloid	National Center for Biotechnology Information, 2006
Chaetochromin			√	$C_{30}H_{26}O_{10}$	547.1577	Heterocyclic	National Center for Biotechnology Information, 2005

Hirsutidin			√	$C_{18}H_{17}O_7$	345.0982	Lipid	National Center for Biotechnology Information, 2005
Quercetin 5,7,3',4'-tetramethyl ether 3-rutinoside			√	$C_{31}H_{38}O_{16}$	689.2065	Flavonoids	Human Metabolome Database, 2012
Haginin A			√	$C_{17}H_{16}O_5$	323.0896	Flavonoids	National Center for Biotechnology Information, 2005
3*Z*,6*Z*,9*Z*,12*Z*,15*Z*-Octadecapentaenoic acid			√	$C_{18}H_{26}O_2$	275.2004	Fatty Acid	National Center for Biotechnology Information, 2006
9,10-Epoxyoctadecatrienoic acid			√	$C_{18}H_{28}O_3$	293.2104	Fatty Acid	National Center for Biotechnology Information, 2008
11-hydroperoxy-12,13-epoxy-9-octadecenoic acid			√	$C_{18}H_{32}O_5$	346.2587	Fatty Acid	National Center for Biotechnology Information, 2006
D-Galactopyranosyl-(1-3)-D-galactopyranosyl-(1-3)-L-arabinose			√	$C_{17}H_{30}O_{15}$	497.1470	Organochalcogen	National Center for Biotechnology Information, 2017
Ciliatin A			√	$C_{20}H_{16}O_5$	337.1063	Flavonoids	National Center for Biotechnology Information, 2009
Epicatechin 5-O-beta-D-glucopyranoside-3-benzoate			√	$C_{28}H_{28}O_{12}$	557.1653	Flavonoids	National Center for Biotechnology Information, 2009
Prosafrinine			√	$C_{17}H_{33}NO_2$	301.2853	Fatty Acid	National Center for Biotechnology Information, 2009
C17-Sphinganine			√	$C_{17}H_{37}NO_2$	288.2906	Organic Amino	National Center for Biotechnology Information, 2014
(-)-Matairesinol 4'-[apiosyl-(1->2)-glucoside]			√	$C_{31}H_{40}O_{15}$	691.2014	Organochalcogen	National Center for Biotechnology Information, 2014
Vitexin 2"-O-rhamnoside 6"-acetate			√	$C_{29}H_{32}O_{15}$	643.1624	Flavonoids	National Center for Biotechnology Information, 2009
(*S*)-Nerolidol 3-O-[a-L-Rhamnopyranosyl-(1-4)-a-L-rhamnopyranosyl-(1-2)-b-D-glucopyranoside]			√	$C_{33}H_{56}O_{14}$	694.4013	Organochalcogen	National Center for Biotechnology Information, 2007
Terminaline			√	$C_{23}H_{41}NO_2$	364.3218	Alkaloids	National Center for Biotechnology Information, 2005
1-(2*E*,4*E*-octadecadienoyl)-sn-glycero-3-phosphocoline			√	$C_{26}H_{51}NO_7P$	520.3395	Fatty Acid	National Center for Biotechnology Information, 2008
5(S)-HETE lactone			√	$C_{20}H_{30}O_2$	303.2311	Fatty Acid	METLIN Database
2-Hydroxyhexadecanoic acid			√	$C_{16}H_{32}O_3$	295.2255	Fatty Acid	National Center for Biotechnology Information, 2005
12-Tricosanol			√	$C_{23}H_{48}O$	379.3348	Fatty Acid	National Center for Biotechnology Information, 2005
1-Monoacylglyceride			√	$C_{21}H_{36}O_4$	353.2681	Fatty Acid	Human Metabolome Database, 2009
N,N-dimethyl-Safingol			√	$C_{20}H_{43}NO_2$	352.3196	Fatty Acid	National Center for Biotechnology Information, 2009

Glycero-3-phosphocholine			√	$C_{46}H_{83}NO_8P$	808.5833	Fatty Acid	National Center for Biotechnology Information, 2011
Phosphoethanolamine			√	$C_{42}H_{82}NO_8P$	760.5827	Fatty Acid	National Center for Biotechnology Information, 2006
1-Oleyl-2-arachidonoyl-sn-glycero-3-phosphocholine			√	$C_{46}H_{83}NO_8P$	808.5867	Fatty Acid	National Center for Biotechnology Information, 2008
1-Monoacylglyceride			√	$C_{27}H_{52}O_4$	441.3920	Fatty Acid	Human Metabolome Database, 2009)
3*E*,13*Z*-Octadecadienyl acetate			√	$C_{20}H_{36}O_2$	309.2781	Fatty Acid	National Center for Biotechnology Information, 2005
Coroloside			√	$C_{35}H_{54}O_{12}$	684.3967	Steroid	National Center for Biotechnology Information, 2005
Coenzyme Q6			√	$C_{39}H_{58}O_4$	613.4219	Organochaclogen	National Center for Biotechnology Information, 2005
(3*S*,5*R*,8*R*,3′*R*)-Mutatoxanthin			√	$C_{40}H_{56}O_3$	585.4295	Terpenoids	National Center for Biotechnology Information, 2007
17,20-dimethyl Prostaglandin F1α			√	$C_{22}H_{40}O_5$	423.2511	Fatty Acid	National Center for Biotechnology Information, 2013
10*Z*,13*Z*-Nonadecadienoic acid			√	$C_{19}H_{34}O_2$	295.2631	Fatty Acid	National Center for Biotechnology Information, 2006

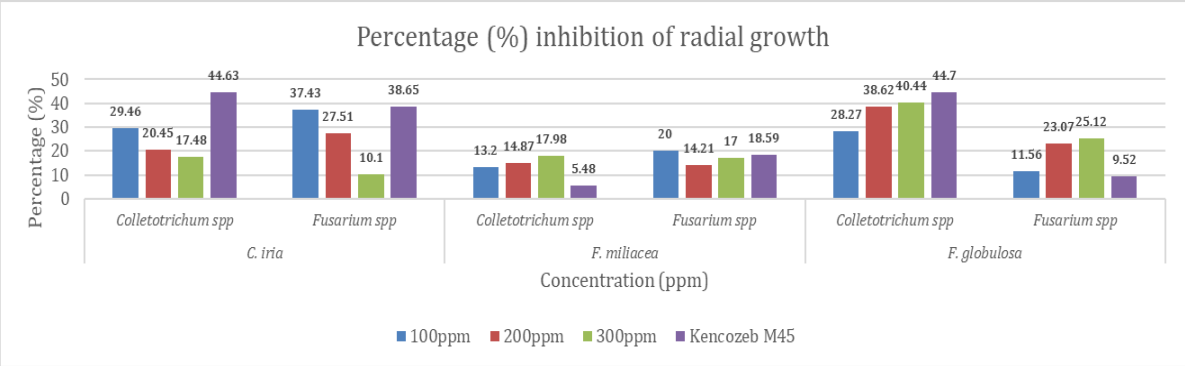

Figure 1. Percentage inhibition of radial growth of *C. iria, F. miliacea and F. globulosa* against *Colletotrichum* spp. and *Fusarium* spp.

Minimum Inhibition Concentration (MIC) and Minimum Fungicidal Concentration (MFC)

The MIC was determined using the broth microdilution method according to the Clinical and Laboratory Standards Institute (CLSI). The inoculum concentration was determined to approximately 10^4 CFU/mL. Sample extracts with concentration of 10 mg/mL were mixed and two-folds diluted in medium containing inoculum. The sample extract containing the highest concentration (5 mg/mL) was placed in Column 12 while the lowest concentration (0.0097 mg/mL) was placed in Column 3. Negative control which consists of only medium with no inoculum and no antimicrobial agent was placed in Column 1. Whereas positive control which consists of sterile broth was placed in Column 2 for 24 hours. The lowest concentration that managed to inhibit microbial growth was defined as MIC. To evaluate fungicidal concentration (MFC), a loop from each concentration which showed the inhibition of the fungus was inoculated onto Potato Dextrose Agar (PDA) and incubated for 5-7 days.

RESULTS AND DISCUSSION

Compounds identification by LC-MS showed 131, 60, and 95 compounds for *C. iria*, *F. miliacea*, and *F. globulosa* respectively (Table 1). Most of the compounds detected were flavonoids and terpenoids. These findings are similar to [12, 13], which stated that there is the presence of terpenoids emitted from leaves part of *C. iria* which have been reported to have antifungal effect on various species [14] and the presence of flavonoids from the root part of *C. iria*. Flavonoids content from *F. miliacea* were supported by previous findings where they possess strong anti-diarrheal effect [15]. Results for *F. globulosa* can be supported by [16] which stated the presence of flavonoids in the methanolic extract of the plant. However, to the best of our knowledge, there is no extensive report on the phytochemical properties of *F. globulosa*.

Results for antifungal activity by agar well diffusion assay showed potential activities towards both Colletotrichum spp. and *Fusarium* spp (Figure 1). The highest value was observed with Kencozeb M45 at 44.63% for *Colletotrichum* spp and 38.65% for *Fusarium* spp. followed by methanolic extract of *C. iria* at 29.46% and 37.43% for both *Colletotrichum* spp. and *Fusarium* spp respectively. The lowest was observed with negative control (distilled water) at 0 for both fungi. Extracts for *F. miliacea* showed potential activities towards both *Colletotrichum* spp. and *Fusarium* spp. The highest value was observed at 300 ppm with 17.98% for *Colletotrichum* spp. and Kencozeb M45 at 18.59% for *Fusarium* spp. respectively. *F. miliacea* extract exhibited the second highest value at 14.87% and 14.21% for *Colletotrichum* spp. and *Fusarium* spp respectively. The lowest value was observed with negative control (distilled water) with no inhibition growth and 12.72% for *Colletotrichum* spp. and *Fusarium* spp. respectively.

The highest value for *F. globulosa* extract was observed with Kencozeb M45 at 44.7% for *Colletotrichum* spp and at 300 ppm of *F. globulosa* extract with 25.12% for *Fusarium* spp. respectively. The lowest value was observed with negative control (distilled water) with no inhibition growth. Both MIC and MFC values were determined by broth microdilution assay (Table 2). The MIC value for *Colletotrichum* spp. was at 125 ug/mL and MFC value at 250 ug/mL for all tested samples. *Fusarium* spp. showed MIC value at 125 ug/mL and MFC value at 250 ug/mL for *C. iria* and *F. miliacea*. *F. globulosa* showed MIC value of 250 ug/mL and MFC value of 500 ug/mL. Antifungal activity against *Colletotrichum* spp. showed MIC and MFC values of 125 ug/mL and 250 ug/mL, respectively. Antifungal activity against *Fusarium* spp. showed moderate to good activity with MIC and MFC value of 250 ug/mL for *C. iria* and *F. miliacea*, respectively, while moderate antifungal activity for *F. globulosa* with MIC value of 250 ug/mL and MFC value of 500 ug/mL.

Table 2. MIC and MFC of tested plant extracts against pathogenic fungi.

Extract	Fungus	MIC (ug/mL)	MFC (ug/mL)
C. iria	*Colletotrichum* spp.	125	250
	Fusarium spp.	125	250
F. miliacea	*Colletotrichum* spp.	125	250
	Fusarium spp.	125	250
F. globulosa	*Colletotrichum* spp.	125	250
	Fusarium spp.	250	500

MIC and MFC of all tested extracts showed moderate to good antifungal activity for all tested samples against both fungi suspension. The antifungal activity for *C. iria* and *F. miliacea* can be attributed with the presence of flavonoids and terpenoids based on the LC-MS analysis and previous studies [12-15]. However, to the best of our knowledge, this is the first study on the antifungal activity of *F. globulosa* against *Colletotrichum* spp. and *Fusarium* spp. Based on previous study by [17], most of the reported national potentiators for antifungal are polyphenols and terpenes. They also stated that there were previous studies that showed the potential of antifungal activity which were related to the presence of secondary metabolites such as terpenes. However, the mechanism on how terpenes work to inhibit the fungal growth is still unknown.

CONCLUSION

Plants produced secondary metabolites in response to the pathogens' attack. Terpenoids and flavonoids played a crucial role in plant defence mechanisms and displayed antimicrobial and antifungal activities [12, 18]. Previous study confirms the potential of antifungal activity of *C. iria*, *F. glubulosa* and F. *miliacea*. However, further research needs to be done to further understand the mechanism of secondary metabolites in Cyperaceae against antifungal activity.

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